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Life Cycle of *Paramecium bursaria* Syngen 1 in a Natural Pond

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ABSTRACT—Studies were made on the natural population density of *Paramecium bursaria* syngen 1, on the life cycle stages to which the individuals belonged, and on the length of the immature and adolescent periods. Green paramecia were collected from a natural pond once every 20 days for two years: 3176 individuals on 37 collection dates. Individuals in nature did not maintain a steady density, but a regular annual change in the population density was not found. Mating tests were conducted on 2955 of 3176 individuals. Of the total, 23 (1%) were adolescent, 21 (1%) were mature, while the others showed no mating reaction. 73 of 315 individuals from two other collections died within two weeks after undergoing 0 to 9 cell divisions. 178 of the other 242 stocks showed fission rates under 1.00. Only 2 stocks showed a rate of over 1.51. Further, 121 of 242 stocks died without expressing mating ability within 4 months after collection. The other stocks lowered their fission rates gradually and often produced abnormal or colorless individuals within stocks instead of expressing mating reactivity. Immature clones from a cross between two stocks originating from the same pond showed fission rates of 1.51 to 2.25. The clones grew to maturity after 38 to 59 cell divisions from clone initiation following an immature period of 36 to 58 cell divisions and an adolescent period of 0 to 17 cell divisions, during which no individuals with abnormal morphology appeared. These results indicate that the natural population in this pond consisted primarily of senile individuals.

INTRODUCTION

The clonal life cycle in ciliates has been analyzed based on the capacity for sexual reproduction, vitality, production of progeny with abnormal micro- and macronuclei or cell shape, and viable progeny following sexual reproduction. Such studies have included *Paramecium bursaria* [21], the *P. aurelia* complex [21, 23], *P. caudatum* [21], *Tetrahymena thermophila* [5, 22], *Euplotes patella* [8], and *E. woodruffi* [9]. In *P. bursaria, T. thermophila, E. patella*, and *E. woodruffi*, immaturity, adolescence, maturity, and senility have been described within the life cycle.

Almost all studies on the life cycle, however, have been done in the laboratory. Only one report of the life cycle in free-living ciliates was made in *Paramecium bursaria* syngen 1: natural populations in streams consisted of immature, adolescent, and mature individuals, but not senile ones. More than one half the individuals of the populations were immature; the appearance of individuals with mating ability appeared to be related closely to increasing population densities. The frequencies of the recessive genes for mating types (*a* and *b*) were higher than for dominant genes (*A* and *B*) [10].

The results obtained from the studies on *Paramecium bursaria* were carried out in two streams where the quality of water was beta-mesosaprobic to oligosaprobic [1, 10]. Would the same results observed in such streams be obtained from different habitats? In other words, does the same species (or syngen more strictly in this case) take the same strategy for survival regardless of its habitat?

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This paper reports mainly field studies, using the holotrichous ciliate *Paramecium bursaria* syngen 1, carried out for two years in a natural pond where the quality of water was alpha-mesosaprobic [1], as well as laboratory work to clarify the following issues: Are there seasonal changes in densities within a natural population? How do frequencies of the four periods in the life cycle change in a natural population? Do individuals with mating capacity appear in certain months or all year? How long in fissions are the periods of immaturity or adolescence?

MATERIALS AND METHODS

Stocks and mating tests on newly-collected stocks

As testers, four mature stocks including stock SJ-2 (mating type I), SH-2 (II), KZ-1 (III), and FD-9 (IV) of *Paramecium bursaria* syngen 1 were used. Two stocks, which were collected in 1991 from Okuda-oike pond in Hiroshima Prefecture, Japan, OK-312 (I) and OK-223 (IV) were used to measure fission rates and the lengths of the immature and adolescent periods. *Paramecium bursaria* (a green *paramecium*) was grown in lettuce infusion containing *Klebsiella pneumoniae* as a food organism at 22–23°C. To avoid aging of paramecia, individuals were isolated and then were allowed to divide only four or five times before making mating tests on each stocks. For green paramecia cultures, we used natural window sunlight rather than artificial lighting. To prevent differences in the peak of mating reaction between tester stocks and newly-collected stocks from nature, we kept both cultures of tester stocks and newly-collected stocks in the same light/dark schedule. Illumination intensity from natural sunlight in the incubator was 1000–1300 lux in the day time. Mating tests were performed during the peak agglutination reactions from 11:30 a.m. to 2:00 p.m. Whenever mating tests were done, high reactivity of mating between the four tester stocks was confirmed. Four to eight cells of a given stock were mixed with 0.3 ml of culture, which included 400–500 individuals of one tester stock. Based on mating reaction, stocks that did not mate with any of the four testers were considered immature; stocks that mated with one or two testers were adolescent; stocks that mated with three testers were considered mature; stocks that did not mate with any of the four testers, but showed low fission rates, or produced colorless (zoochlorellae-free) or abnormal individuals, were termed senile.

Field study site and a method for collecting green paramecia

After almost three years of preliminary investigation to select the field study site, we chose Okuda-oike pond, located in Hiroshima Prefecture, Japan. This pond has an abundance of water plants and is alpha-mesosaprobic without the possibility of drying up and without artificial pollutants from homes and industries. Six stations were established (Fig. 1). The studies took place once every 20 days from October, 1987 to October, 1989. Field samples of green paramecia were collected and isolated by the method which we have already reported [10].

Fig. 1. Sampling stations in Okuda-oike pond that is about 3 m at the deepest and has an area of 0.03 km^2 Water depth is 30 cm in station A; 15–20 cm in B, C, D, and E; 35–40 cm in F. Many leaves of water plants such as *Brasenia schreberi* and *Nymphaea tetragona* which cover the surface of the water are seen. White scale bar (arrow) is 1 m in length.

Fission rates and length of immature and adolescent periods

To measure fission rates, newly-collected green paramecia from nature, which were established from two times of collection in April, 1991, were used. The paramecia were washed three times with an exhausted medium of *P. caudatum* on the same day that water samples were gathered. Each green paramecium was cultured for two days in a medium which consisted of 1 part of the exhausted medium and 1 part of bacterized culture medium. Afterwards, one of the paramecia proliferated was transplanted to a fresh bacterized culture medium. The transplantation of the paramecium was made every 2 days. Fission rates were obtained by calculating the total number of fissions of a clone in two weeks divided by 14. Fission rates and the length of the immature and adolescent periods of descendant clones were also measured by 2 day period isolation culture. Stocks OK-312 and OK-223 were used as parental ones. All the newly-isolated stocks, descendant clones, and tester stocks were maintained and the experiments were made under the 10L/14D artificial lighting condition (4000–5000 lux).

Fixation and staining

Materials were fixed with a fixative solution consisting of 5 parts of saturated mercury chloride, 1 part of glacial acetic acid, 1 part of formalin, and 5 parts of t-butanol [2]. The nuclei were stained with Delamater's basic fuchsin for permanent staining [14]. Hydrolysis was done in 4N Hcl for 30 min at room temperature.

RESULTS

Population variations

Thirty-seven collections were done from October 13, 1987 to October 18, 1989. The results for population fluctuation of green paramecia are shown in Table 1 and Fig. 2. The 3176 green paramecia in samples collected from 6 stations in Okuda-oike pond were isolated. Because the population size did not vary among stations (e.g. 497 individuals from station C at the least to 572 from station A at the most), there may not be differences in the microhabitats that green paramecia prefer. Or they may prefer some, but this pond has a relatively uniform habitat.

Figures 2 and 3 show how the population density of green paramecia varied throughout the year. No periodic changes were seen. The population size did not decrease when water temperatures exceeded 20°C or even 30°C.

Frequency of immature, adolescent, mature, and senile stocks in a natural population

In the total of 37 collections, 3176 green paramecia were found and 2955 of them grew to stocks. These were examined by careful mating tests to determine to which period in the life cycle they belonged (Table 1), including mating types for mature stocks. Of 2955 stocks examined in detail, only 44 stocks showed mating capacity: 23 stocks (1%) were adolescent, and 21 stocks (1%) were mature. The others did not mate with the testers. It was not determined clearly whether the non-mating stocks belonged to the immature or to the senile life cycle stages. However, since most of them showed low fission rates (under 0.5), these stocks seemed to be senile. Also 567 of 3176 individuals which died before mating test or showed fission rates of about 0.1 might be senile individuals. The natural population seemed to consist mostly of senile individuals; Only 1% of individuals collected had mating capacity. Four mating types (I to IV) appeared at about the same rate among mature stocks.

From twelve collections, 23 adolescent stocks were isolated (Table 2). The adolescent stocks that did not mate with two or three testers could be divided into 10 subtypes. Fourteen of 23 stocks did not mate with two testers; the other 9 stocks did not conjugate with three testers.

Fission rates, short-lived stocks, and appearance of individuals with abnormality

Many individuals which seemed to be senile were found in the collections all through the two-year study. To examine in detail whether these individuals were truly senile, two more collections were done on April 3rd and 21st, 1991.

TABLE 1. Number of non-mating and mating individuals

Date			No. of paramecia tested							No. of paramecia not tested		
			Non-mating	Mating						Slow growing*	dead**	
				Adolescence	Maturity Mating types							
					I	II	III	IV				Total
1987	Oct.	13	72	0	0	0	0	0		11	5	77
	Nov.	4	22	0	0	0	1	0		5	0	23
	Nov.	22	26	0	0	0	0	0		2	3	29
	Dec.	15	45	0	0	0	0	0		6	5	50
1988	Jan.	6	61	0	0	0	0	0		4	7	68
	Jan.	26	211	0	0	0	0	0		15	12	223
	Feb.	16	59	0	0	0	0	0		1	0	59
	Mar.	6	14	0	0	0	0	0		1	2	16
	Mar.	25	29	1	0	0	0	0		5	2	32
	Apr.	14	77	3	1	0	0	2		9	6	89
	May	6	74	0	0	0	0	0		2	3	77
	May	26	64	0	0	0	0	0		9	3	67
	Jun.	15	80	0	0	0	0	0		13	11	91
	Jul.	6	163	0	0	0	0	0		17	18	181
	Jul.	26	174	0	0	0	0	0		5	11	185
	Aug.	15	94	0	1	0	0	0		10	5	100
	Sep.	4	107	0	0	0	0	0		18	4	111
	Sep.	25	123	3	0	0	1	0		19	10	137
	Oct.	17	77	0	0	0	0	0		14	11	88
	Nov.	7	30	0	0	0	0	0		5	11	41
	Nov.	25	66	3	0	1	0	0		13	9	79
	Dec.	15	86	0	1	3	1	0		7	5	96
1989	Jan.	4	110	3	1	2	0	2		2	4	122
	Jan.	25	160	3	0	0	1	0		17	8	172
	Feb.	16	60	1	0	0	0	0		11	8	69
	Mar.	7	98	2	0	0	1	0		19	4	105
	Mar.	28	105	0	0	0	0	0		24	5	110
	Apr.	18	86	0	0	0	0	0		6	5	91
	May	9	101	1	0	0	0	0		15	1	103
	May	28	33	0	0	0	0	0		4	1	34
	Jun.	17	139	1	0	0	0	0		6	1	141
	Jul.	6	28	0	0	0	0	0		10	14	42
	Jul.	27	55	0	0	0	0	0		23	22	77
	Aug.	17	48	0	0	0	0	0		3	1	49
	Sep.	6	57	1	0	0	0	1		5	0	59
	Sep.	27	43	1	0	0	1	0		4	1	46
	Oct.	18	34	0	0	0	0	0		6	3	37
Total			2911	23	4	6	6	5		346	221	3176

* Slow growing individuals which underwent only one or two cell divisions over ten days.
** Individuals died within ten days after isolation.

From these collections, 315 individuals were isolated.

The individuals collected were cultured by 2-day period isolation for two weeks. Seventy-three of 315 individuals died after undergoing 0 to 9 cell divisions within the tèrm (Table 3). Fifty-four (74%) of 73 individuals died without cell division or after only one cell division. The other 242 individuals grew to stocks. They were transfered to fresh culture medium once every ten days and were cultured in Petri dishes 3.5 cm in diameter for four months and were examined about features of the life cycle.

Table 4 shows the stocks which died within four months. Although mating tests were done several times, 194 (80%) of

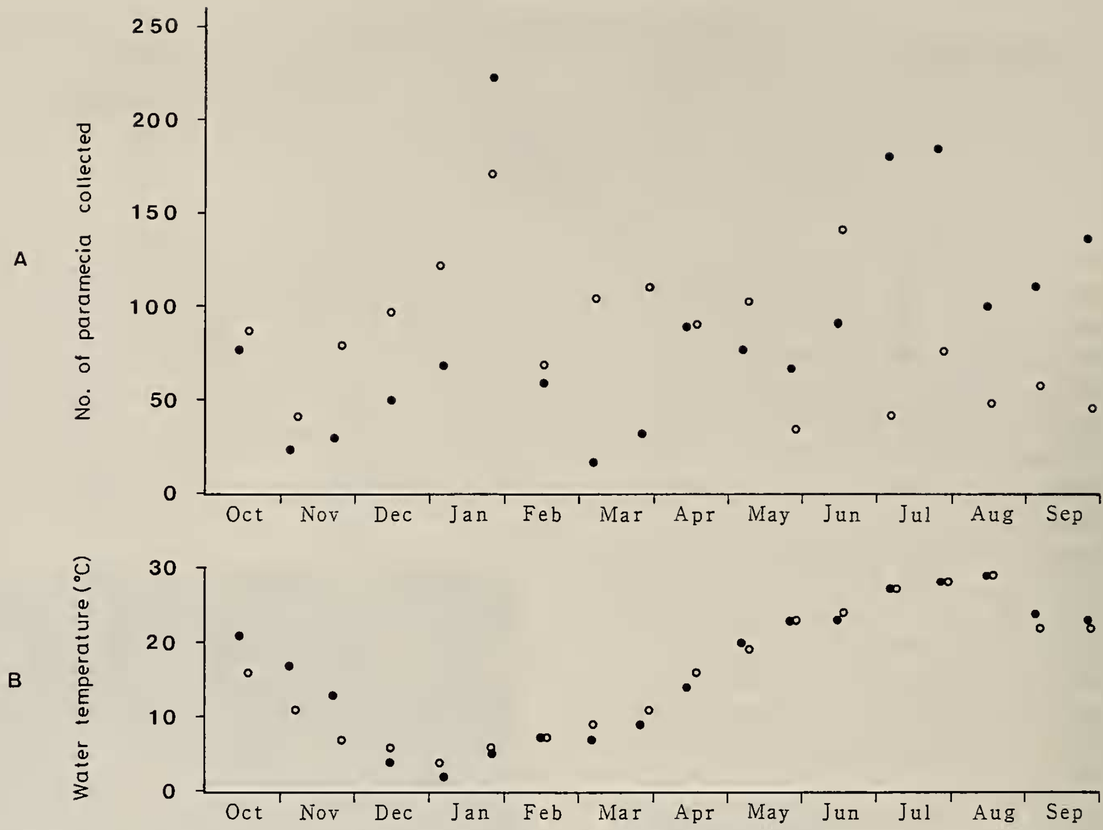

FIG. 2. Number of individuals collected in a Okuda-oike population (A). Water temperature (B). ●—●, 1987–1988; ○—○, 1988–1989.

the 242 stocks died without showing any mating reactivity with the testers. The other 48 stocks lived for over four months, but they showed no symptom of mating during the 4-month culture period except one stock.

Table 5 shows fission rates of the stocks which survived for over two weeks. 178 (74%) of 242 stocks showed fission rates under 1.00. Two stocks with the fission rate over 1.51 were mature, and belonged to mating type I or IV.

Of the stocks which did not react with the testers just after collection, only one stock began to express mating capacity during the 4-month culture period. Many of the other stocks died one by one instead of expressing mating capacity. Further, individuals with various abnormalities such as small individuals (Fig. 4, b, c), an amicronucleate (Fig. 4, d), a bi-micronucleate (Fig. 4, e), colorless individuals (Fig. 4, f, g), individuals with unusual shapes and nuclei (Fig. 4, h, i, j, k), and connected individuals (Fig. 4, l, m) appeared in the stocks. In 84 (35%) of 242 stocks which lived for over two weeks, individuals with some abnormality appeared. The various stocks might exhibit only one type of abnormality, or two or more types of abnormalities.

Fission rates and the length of immaturity and adolescence

The results obtained indicated that there might be many senile individuals in a natural population. To ascertain this possibility more clearly, it is necessary to provide further evidence that the individuals likely to be senile show quite different features from young individuals. Two remaining main issues to be re-solved are the following: How many times do young individuals divide per day?; How long is the immaturity period of the individuals?

Two stocks, OK-312 (I) and OK-223 (IV), were used as parental stocks. Using descendant clones from a cross between the parental stocks, fission rates and the lengths of immaturity and the adolescence were studied.

The viability of exconjugants was 99% when 100 exconjugants from 50 pairs were examined. 16 clones (8 synclones) of the exconjugant clones were randomly chosen, and were maintained by 2-day period isolation culture. Fission rates of the clones are shown in Table 6. All clones, which were in the immature period, showed fission rates over 1.50.

These clones were kept in 2-day period isolation culture, and were examined to determine the lengths of the immature and the adolescent periods. The results are shown in Figure 5. The shortest immature period was 35 fissions, while the longest was 57 fissions. Adolescence, which was 4 to 17 fissions in length, was observed in 9 of 16 clones, but not in the others. All the clones became mature immediately after the immature (or adolescent) period. When they became mature, each clone yielded steady aggultination reactions with three of four testers when mixed together. However, clones that had just become mature often did not produce conjugating pairs with the testers following the aggultination reaction. All four mating types (I to IV) arose in the

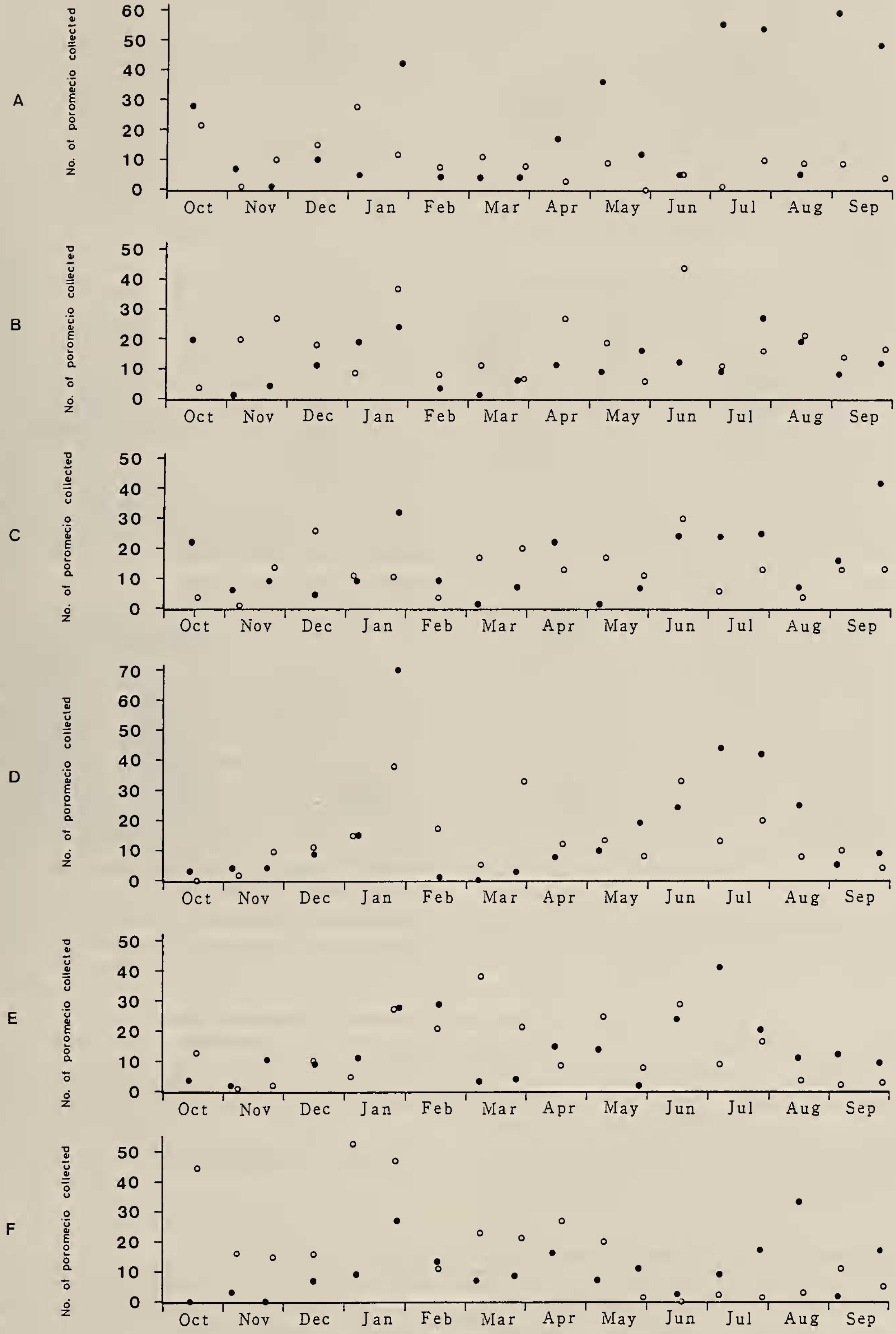

FIG. 3. Number of individuals collected in station A to F. ●—●, 1987–1988; ○—○, 1988–1989.

Table 2. Number of adolescent stocks classified into ten different types

Date		No. of adolescent paramecia — No mating reaction with: I II	I III	I IV	II III	II IV	III IV	I II III	I II IV	I III IV	II III IV	Total
1988	Mar. 25						1					1
	Apr. 14	1				2						3
	Sep. 25	2		1								3
	Nov. 25	1						1		1		3
1989	Jan. 4						1		1		1	3
	Jan. 25			1				1			1	3
	Feb. 16	1										1
	Mar. 7				1		1					2
	May 9		1									1
	Jun. 17							1				1
	Sep. 6								1			1
	Sep. 27							1				1
Total		5	1	2	1	2	3	4	2	1	2	23

Table 3. Length of life of the short-lived stocks

No. of cell divisions	No. of paramecia — Days: 0–2	3–4	5–6	7–8	9–10	11–12	13–14	Total
0	1	27	2	1			1	32
1		11	6	4	1			22
2				3				3
3				3				3
4				1	2	1	1	5
5						2		2
6							1	1
7					1	1		2
8								0
9						1	2	3
Total	1	38	8	12	4	5	5	73

descendant clones. The mating types were determined synclonally. Individuals with abnormal morphology never were observed in the clones during 100 or more fissions after clone initiation.

DISCUSSION

Individuals of *Paramecium bursaria* appear through the entire year and seasonal changes in their population densities are not observed in Okuda-oike pond where the quality of water is alpha-mesosaprobic. It is unclear whether individuals with mating ability appear only in certain months of a year. By contrast, *P. bursaria* living in streams, where the quality of water is oligosaprobic to beta-mesosaprobic, show seasonal changes in population density and a close relationship between the increasing of population density and the appearance of individuals with mating ability [10]. The density of individuals contained in 600 ml of water samples,

Table 4. Number of the stocks which died within 4 months after isolation

	Length of life: 1 day-14 days	15 days-1 month	1 month-2 months	2 months-3 months	3 months-4 months	Total
No. of stocks	73	41	30	24	26	194

Table 5. Fission rates of the stocks isolated from nature

	Fission rates: 0–0.25	0.26–0.50	0.51–0.75	0.76–1.00	1.01–1.25	1.26–1.50	1.51–1.75	Total
No. of Stocks	3	4	43	128	58	4	2	242

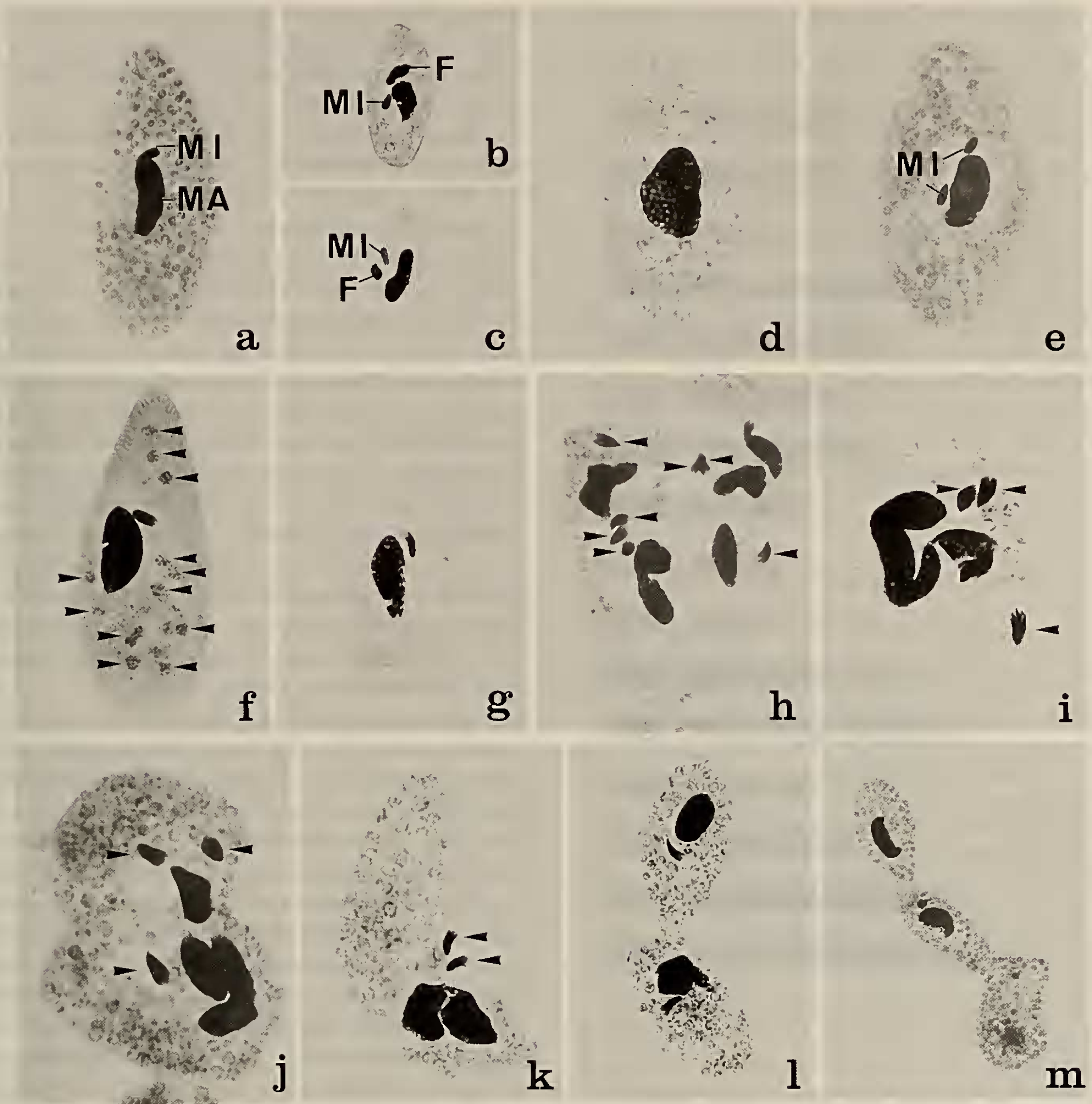

FIG. 4. A normal individual with one micronucleus(MI) and one macronucleus (MA) in which many zoochlorellae are uniformly distributed, ×270 (a). Small individuals with extra macronuclear fragments (F), ×270 (b, c). An amicronuclear individual, ×410 (d). A bimicronuclear individual, ×330 (e). A colorless individual. Several zoochlorellae (arrowhead) are still seen, ×330 (f). A colorless individual without zoochrorellae, ×360 (g). Abnormal and irregular-shaped individuals in which two to five macronuclei and two to seven micronuclei(arrowhead) are seen (h-k): ×360 (h); ×570 (i); ×430 (j); ×410 (k). Connected individuals (l, m): ×290 (l); ×170 (m).

TABLE 6. Fission rates of the immature clones from a cross between stock OK-312 and stock OK-223

	Fission rates				
	0–1.50	1.51–1.75	1.76–2.00	2.01–2.25	Total
No. of clones	0	5	8	3	16

which is obtained by calculating the total number of individuals divided by the total number of collections, is 15.9 in Mikumarikyo stream and 3.6 in Momijidanigawa stream [10], while 85.8 in Okuda-oike pond. Thus, many more individuals are contained in the same volumes of water samples in a pond than those in a stream. The results show that even the same species (syngen) might take different survival strategies in different habitats.

A period of adolescence has been reported in *Paramecium bursaria* [6, 16], *Tetrahymena thermophila* [17], *Euplotes patella* [8], *E. woodruffi* [9], and *E. octocarinatus* [12] which are regarded as outbreeding species. Clones with or without a period of adolescence were observed in the descendant clones of *P. bursaria* in Okuda-oike pond. The duration of adolescence was only 17 fissions at the most. The appearance of clones without adolescence is the first case so described in *P. bursaria*. Because the adolescent period is short or lacking, and the immature period short, it can be

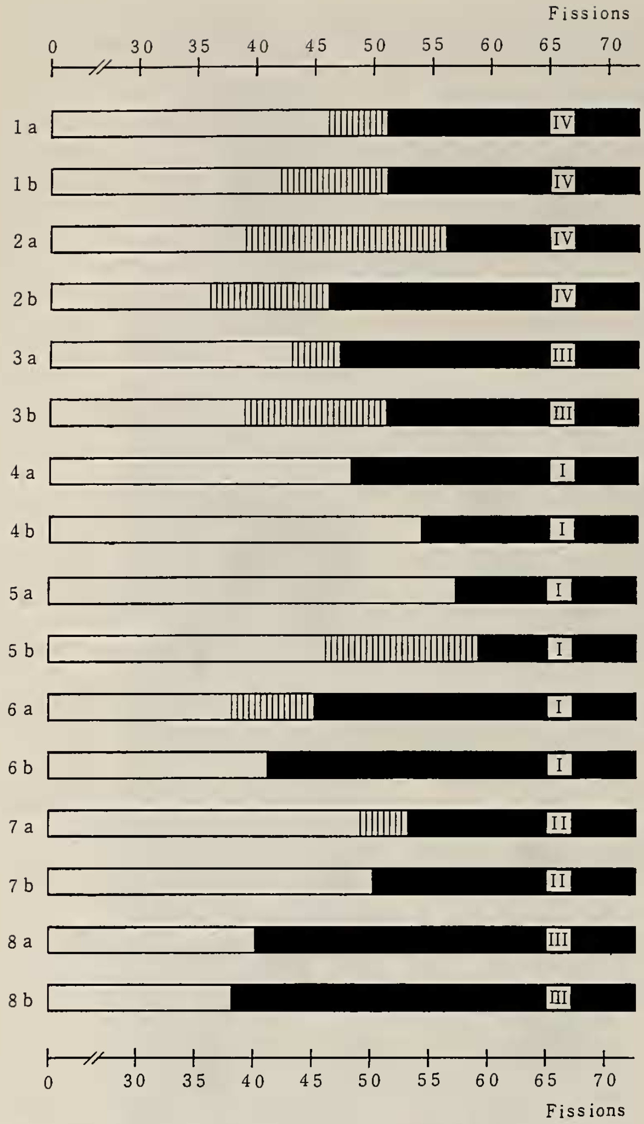

Fig. 5. Length of the immature and the adolescent periods. □ immature period; ▥ adolescent period; ■ mature period. Roman numerals in the figure mean the mating types to which F1 clones were differentiated. Each pair of clones (1a and 1b, for example) derived from a single synclone.

suggested that green paramecia take an inbreeding strategy in Okuda-oike pond.

The field studies on the life cycle for two years in Okuda-oike pond reveal that individuals with mating ability, which are adolescent individuals plus mature ones, were only 1% of the total individuals collected. The rate is very much lower than that found in a Mikumarikyo population (25%) and a Momijidanigawa population (47%) [10]. Furthermore, collections from Mikumarikyo and Momijidani-gawa populations revealed no senile individuals, whereas the Okuda-oike population consisted of many individuals with very low fission rates (under 0.5) and short-lived individuals. The data indicate that at least the 346 (11%) with low fission rates and 221 (7%) short-lived individuals of 3176 individuals probably belong to the senile period. Several features of senility have been reported: decline in fission rates is known in *Paramecium aurelia* [20], *P. caudatum* [24], and *E. woodruffi* [9]; cytological abnormality is in *P. aurelia* [4, 15, 21], *P. caudatum* [24], and *E. woodruffi* [9]; macronuclear abnormality is in *P. aurelia* [4], *P. caudatum* [3, 24], *E. patella* [8], and *E. woodruffi* [9]; micronuclear aberration or loss is in *Tetrahymena pyriformis* [25], and *P. aurelia* [4, 15, 21].

Again, the results from the later two times of collection show that the same population consists of many senile individuals. Of 315 individuals collected, 194 (62%) died within four months. Because more than half of the individuals died without expressing mating ability with advancing age, they must be considered to be senile. Of 242 stocks which lived for over two weeks, 178 (74%) showed fission rates under 1.0. Because all immature clones showed fission rates over 1.5, those individuals with fission rates under 1.0 would appear to be senile. Further, in 84 (35%) of 242 stocks, various abnormalities such as small-sized or colorless individuals, or cytological abnormalies in body shape and micro- and macronuclus, were observed. The appearance of individuals with abnormalities in the stocks indicates that those stocks also were senile.

It was reported that the life span of *Paramecium bursaria* was 2585 days [7]. If a green paramecium divides once a day, the length of life calculated in terms of cell divisions is about 2600 cell divisions; if it divides 0.5 a day, it is 1300 cell divisions. If we assume that the length of maturity of green paramecia is one half on the life-span, it would last from 650 to 1300 cell divisions. On the other hand, many senile individuals were most probably included in the Okuda-oike population. If members of the population have the same life span as the former report, they could naturally encounter and mate with mature individuals of complementary mating types during this long period. However, in reality this seems not to occur in Okuda-oike pond. Actually, the length of immaturity which was reported to last over one year [21] is only 36 to 57 cell divisions. A short immaturity period for *P. bursaria*, about 50 fissions, was briefly reported by Siegel [18]. Further, adolescence is short or even absent, and the life span is 151 to 281 cell divisions (171 to 260 days) in the Okuda-oike population (Kosaka, unpublished). These facts present the likelihood that the members of the Okuda-oike population are destined to reach the period of senility even more quickly. Because of three factors: (1) appearance of individuals with mating ability appear throughout the year, not concentrated in certain months, (2) only a few individuals show mating ability at a given time in a population, and (3) mature individuals show a short period of maturity, cells in this pond might have aged by missing chances to encounter and mate with individuals of complementary mating types.

The question arises why so many senile individuals which are already dead genetically continue to live in the pond population? The first answer to the question is that water flow might play an important role in removing senile individuals from the population, because senile individuals that lower their physiological activities have never been observed in streams [10]. Since senile individuals are found in a pond where water current is very slow but not in streams, they might be carried away more easily than young individuals. The second answer is that green paramecia have symbiotic zoochlorellae in the cytoplasm [11, 26, 27]. The presence of zoochlorellae allows green paramecia to live even with depressed physiological activity, because nutrient is supplied to green paramecia from their zoochlorellae. The third answer is that the quality of water of the pond chosen for the present study is alpha-mesosaprobic. As expected, more bacteria as food organisms must be contained in the water of alpha-mesosaprobic than that of oligosaprobic and beta-mesosaprobic, because the former contains more organic substance. For these three reasons, even senile individuals of *P. bursaria* might be capable of living in a natural pond.

All four mating types (I-IV) were collected in Okuda-oike pond from the two-year collection (21 stocks) and the additional two times of collection (2 stocks): 5 stocks belonged to mating type I; 6 stocks, II; 6 stocks, III; 6 stocks, IV. The mating types of *Paramecium bursaria* syngen 1 are determined by the combination of dominant (*A* or *B*) or ressessive (*a* or *b*) alleles of two nonlinked loci [19]. Genotypes of mating type I are *AABB, AABb, AaBB,* and *AaBb*. The genotype of mating type III is the double recessive homozygote *aabb* while genotypes of mating types II and IV are recessive homozygote for one of two alleles (*aaBB* or *aaBb* and *AAbb* or *Aabb*, respectively). Frequencies of *a* and *A* or *b* and *B* can be calculated as follows: 12 of 23 mature stocks collected from Okuda-oike pond that belong to mating type II and III have recessive genes *aa*. The frequency of *aa* is obtained by dividing 12 by 23: therefore, the frequency of gene *a* is $\sqrt{12/23}=0.72$. Thus, the frequency of the *a* allele is 72% and the *A* allele is 28%. On the other hand, 12 of 23 mature stocks, which belong to mating type III and IV, have recessive genes *bb*. The frequency of *bb* is obtained by dividing 12 by 23: therefore the frequency of *b* is $\sqrt{12/23}=0.72$. The frequency of the *b* allele is 72%, and the *B* allele is 28%. The finding that the frequencies of the recessive alleles for mating types are higher than for dominant alleles in a pond population is very similar to the results obtained from stream populations [10]. This indicates that members of both populations increase their mating chances by decreasing in the frequencies of the dominant mating type alleles [10].

Mating type determination was synclonal in the descendants from a cross between two stocks from Okuda-oike pond. The results agree with those of Jennings [7]. According to Sonneborn, the usual synclonal uniformity in mating type serves to reduce the probability of mating between close relatives [21]. However, our results do not lead to the same conclusion. The first reason is that four mating types, which are determined synclonally, appeared in the decendant clones from the same cross. The results present the possibility that matings occur between close relatives. The second reason is that the frequencies of recessive alleles are three times higher than those of dominant ones in this pond. This means that the same cross can more easily yield more than one mating type. For example, let us consider the case when mating occurs between different stocks of mating type I and III. Mating between genotypes *AABB* (mating type I) and *aabb* (III) yields only genotype *AaBb* (corresponding to mating type I). However, mating between genotypes *AaBb* (I) and *aabb* (III), produces all four mating types at the same rate: genotypes produced will be *AaBb* (mating type I), *aaBb* (II), *aabb* (III), and *Aabb* (IV). For these two reasons, it is probable that sib-cross as well as out-crosses might occur in places where the frequencies of recessive alleles are higher than those of dominant ones. Further, the data indicate that *Paramecium bursaria* syngen 1, which has been considered one of the typical outbreeders [13, 21], adopts an inbreeding strategy when found in a pond environment.

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